

# The Mother Lode of Liver Transplantation, With Particular Reference to Our New Journal

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With certification for inclusion in the *Index Medicus*,\* *Liver Transplantation and Surgery* will enter an exponential phase of its development. Authors contributing to the Journal now will find their work accessible to literature search via the same informational panoply as other major publications. The Journal has obtained an additional edge by its designation as an official organ of the American Association for the Study of Liver Disease (AASLD). However, its most important advantage is the richness of the material to which *Liver Transplantation and Surgery* has potential access.

The reports about clinical liver transplantation that have filled the pages of the Journal until now constitute only the small tip of a large biological iceberg. Each member of the abdominal organ complex (Fig. 1) has anatomical and functional relationships with all the others. Probing into these interactions during the evolution of liver transplantation set off ripple effects at least as important for continuing creative research and development as the kind of minor refinements, case studies, and expressions of opinion about the procedure itself that quickly reach the point of diminishing returns. The argument can be made best with historical examples that continue to foster inquiry.

## Portal (Hepatotropic) Physiology

The possibility of transplanting the whole liver was first mentioned in the scientific literature in 1955 by C. Stuart Welch of Albany, NY.<sup>1</sup> Envisioning potential clinical application, Welch described the insertion of an auxiliary hepatic allograft into the right paravertebral gutter or pelvis of nonimmunosuppressed dogs (Fig. 2A). When the portal venous inflow was derived from the inferior vena cava as shown, the auxiliary grafts underwent rapid atrophy. Welch's misconception that the shrinkage was a manifestation of unmodified rejection<sup>1,2</sup> was not corrected until his experiments were repeated under effective immunosuppression.<sup>3</sup> Although rejection

could be prevented, the atrophy was essentially the same.

It was then realized that there were liver-supporting constituent(s) of splanchnic venous blood that were not available to the graft because they were efficiently removed with the first pass through the native liver.<sup>3</sup> Histopathologically, the shrunken auxiliary liver deprived of splanchnic venous blood was indistinguishable from that caused in rats, dogs, and primates by portacaval shunting (Eck's fistula) (summarized in reference 4).

Because it was difficult, and sometimes technically impossible, to divert splanchnic venous blood to an auxiliary liver, these observations pushed the pendulum of the early 1960s toward the ostensibly more radical operation of liver replacement. However, it was learned at the outset that a single (orthotopic) liver allograft also required normal portal revascularization for optimal results<sup>5</sup> (Fig. 3). The same principle applies today. If orthotopic liver transplantation is performed in patients who have portal-systemic shunts, the shunts should be disconnected. In patients who have thrombosis or damage to their native portal veins, extension or jump grafts can be used to ensure delivery to the transplanted liver of the hormone- and nutrient-rich splanchnic blood.<sup>6-9</sup> Alternative procedures that augment portal flow by portal arterialization or transhepatic rerouting of venous blood (i.e., portacaval transposition) can improve circumstances, but the resulting liver physiology is not normal.

## The Mysterious Eck Fistula

In 1961, Bollman,<sup>10</sup> the world's leading authority on "meat intoxication" (hepatic encephalopathy)

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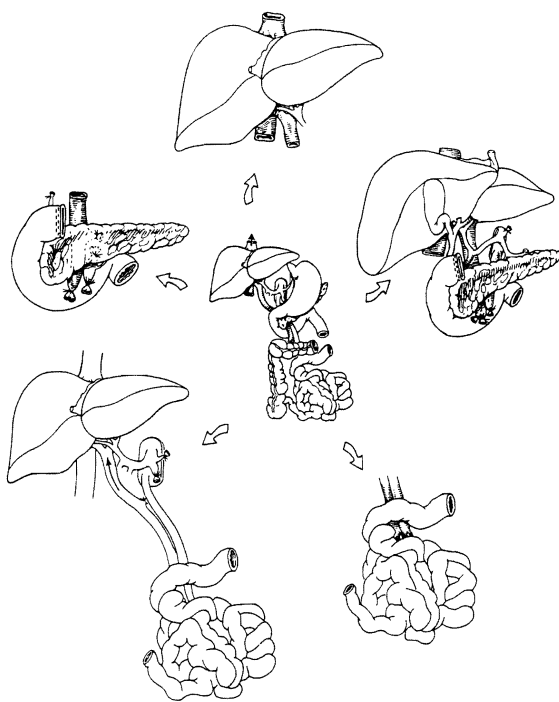
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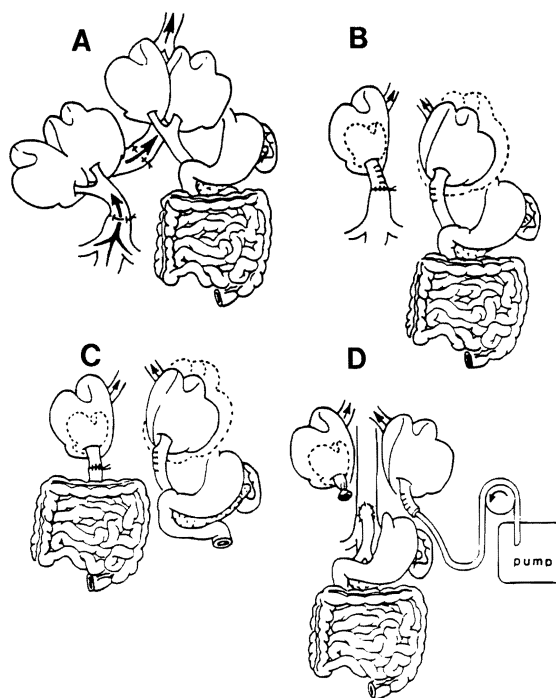
**Figure 1. The complex of intraabdominal viscera (center) functional and anatomic interrelations. (Reprinted with permission from *Transplant Proc* 1996;28:2430-2432.)**

and other complications of portacaval shunting, wrote: "In the 83 years since it was first reported, the Eck fistula has been reasonably successful in hiding its secrets as well as giving rise to many additional questions fundamental to an understanding of the functions of the intestine, liver, and brain."<sup>10</sup> These previously enigmatic syndromes became comprehensible when insulin finally was proved in 1975 to be the most important hepatotrophic factor in splanchnic venous blood.<sup>4,11,12</sup> Until then, the entrenched dogma of hepatic physiology had been that the volume rather than the source of portal venous blood flow was the critical factor in hepatic homeostasis and control of liver mass (the flow hypothesis<sup>4</sup>). Now it was evident that the principal liability of the splanchnic diversion procedures came from their placement of the liver in an insulinoprival state.

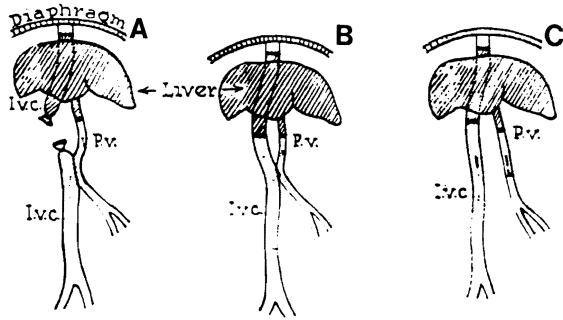
The partial amelioration of Eck fistula morbidity by flow augmentation procedures such as Child's portacaval transposition<sup>13</sup> or Fisher's portal arterIALIZATION<sup>14</sup> was explained. With these strategies, diluted insulin and other splanchnic hepatotrophic substances recirculated in systemic blood were made more available to the liver in proportion to

the increased total hepatic blood flow. The double liver models, of which auxiliary liver transplantation was the prototype (Fig. 2), were crucial to this understanding. The well-known efficiency of insulin removal during the first pass through the liver made this and other visceral hepatotrophic molecules relatively unavailable for the allograft and thus exaggerated the effect of portal vein deprivation.<sup>15-20</sup>

It was readily understood why total splanchnic venous diversion (i.e., portacaval shunting) was such an acute insult to the already damaged liver of patients with hepatic disease, particularly when there had been significant portal flow before. Although the identity of "liver-supporting substances" in portal blood was not known until a dozen years after the original auxiliary transplantation investigations, the circumstantial evidence



**Figure 2. Double-liver and double-liver fragment models that have been critical for the elucidation of splanchnic hepatotrophic factors: (A) auxiliary liver transplantation; (B) partial portacaval transposition, separating the effect on the liver of systemic versus splanchnic portal inflow; (C) splanchnic venous division, separating the effect of venous return from upper and lower abdominal viscera; and (D) Eck fistula plus selective infusion of test substances to one or the other of 2 liver fragments. (Reprinted with permission from *Hepatology* 1994;20:747-757.)**



**Figure 3. Methods of venous reconstruction evaluated in 1958 through 1960 for canine orthotopic liver transplantation. (A) Reverse Eck fistula; (B) anatomic reconstruction with portacaval shunting; (C) completely anatomic portal venous reconstruction. Optimal results were obtained only in C. (Reprinted with permission from Surg Gynecol Obstet 1960;111:733-743.)**

that these molecules were present in splanchnic venous blood explained the advantage of selective portal diversion procedures such as the Warren shunt<sup>21</sup> that included a lower postoperative incidence of encephalopathy and liver failure.<sup>22</sup>

Until this time, most surgeons and hepatologists had assumed that the normal human liver functioned essentially normally after portacaval shunting except for hyperammonemia.<sup>23,24</sup> This view was inconsistent with the striking hepatocyte atrophy (to half size), deglycogenation, and fatty infiltration demonstrated in both Eck fistula and double liver models during the 1960s and 1970s<sup>4,12,20</sup> in all species studied, including baboons<sup>25</sup> and humans.<sup>4,26</sup> At the same time, there is a tripling of liver cell renewal.<sup>4,11,12,25,27</sup> Both the atrophic and hyperplastic alterations caused by the portaprival state are complete within 4 days in dogs and remain stable thereafter (Table 1).<sup>11,12,18,20</sup>

The ultrastructure of the hepatocytes of the

portaprival liver shows a striking disruption of the rough endoplasmic reticulum and depletion of the ribosomes,<sup>11,12,28,29</sup> explaining a reduced synthesis of cholesterol and other lipid moieties, bile acids, urea, and presumably essentially all metabolites of hepatic origin (summarized in reference 4). Decreased activity of the hepatic microsomal mixed function enzyme system for which multiple cytochrome P-450 and P-448 species serve as terminal oxidases accounts for other subtle but cumulatively massive degradations in hepatic function.<sup>4</sup>

### *Treatment of Liver-Based Inborn Errors of Metabolism*

#### **With Portacaval Shunting**

Historically, portal diversion procedures were done only to treat complications of portal hypertension (variceal hemorrhage and ascites),<sup>4,22</sup> or rarely to extend the resection margins of hepatobiliary-pancreatic neoplasms.<sup>23,24</sup> Their use was now extended to the palliation of three congenital inborn errors of metabolism: glycogen-storage disease,<sup>30,31</sup> familial hypercholesterolemia,<sup>26,32</sup> and  $\alpha_1$ -antitrypsin deficiency.<sup>33,34</sup> Because each of these errors can be corrected far more effectively by liver transplantation,<sup>35-39</sup> their treatment with portacaval shunting has been abandoned. Between 1965 and 1980, however, shunt surgery was the safest therapy available.<sup>4</sup>

The governing concept with glycogen-storage disease and  $\alpha_1$ -antitrypsin deficiency was that portacaval shunting selectively damaged the rough endoplasmic reticulum, where the synthetic processes of the liver are concentrated, without a commensurate diminution in hepatic excretory function or in reduction of resorption of the abnormal metabolic deposits. There was a similar mechanism in familial hypercholesterolemia. The palliation in this disease came from the >80% reduction in hepatic lipid synthesis caused by portacaval shunting in rats, dogs, baboons, and humans (summarized in references 4 and 32). In patients with homozygous familial hypercholesterolemia, the result was a decline by a third to a half or more of the astronomical plasma cholesterol and low-density lipoprotein (LDL) levels (1,000 mg/dL).<sup>26,32</sup> Without treatment, these patients die in their teens or early 20s of coronary atherosclerosis and other complications of premature atherosclerosis.

**Table 1. Evolution of Changes in the Liver After Eck Fistula Surgery**

Time (d)	Labeled Hepatocyte/1,000	Cell Size Units
0	1.5	0.18
1	1.5	0.17
2	1.9	0.15
3	3.2	0.12
4	4.5	0.09
60	4.5	0.09
Composite data from references 11, 12, 19, and 28.		

### The Shift to Liver Replacement

The rationale was obvious for using liver replacement rather than portacaval shunting to treat the principal glycogen-storage diseases and  $\alpha_1$ -antitrypsin deficiency. However, liver replacement was not used successfully for any inborn error until the correction of Wilson's disease<sup>40,41</sup> in the early 1970s. Nevertheless, the biochemical basis for case selection had begun to be laid in 1956, when structural abnormalities of metabolites and especially deficient enzymes that regulate the production or elimination of gene products were used for the first time to define clinical disorders and determine which were "liver based." The first to be defined biochemically was glycogen-storage disease.<sup>42</sup>

Meanwhile, it was learned in the 1960s that although the nonparenchymal "passenger leukocytes" of the orthotopically transplanted liver were promptly replaced by those of the recipient,<sup>43</sup> the hepatocytes remained of donor phenotype and permanently endowed the recipient with the donor's metabolic profile.<sup>3,43,44</sup> Armed with this information, compilation began of the hepatic-based inborn errors that could be cured with liver replacement. The list of diseases successfully treated with transplantation by 1989 had grown to more than two dozen.<sup>38</sup>

In some of these disorders, however, the transplantation itself became the means by which the liver's role was clarified. Familial hypercholesterolemia was a prime example, and a highly controversial one. Because we had demonstrated that end-to-side portacaval shunting caused a marked decrease in blood cholesterol levels of normal dogs,<sup>20</sup> and that the same thing occurred in patients with glycogen-storage disease<sup>31</sup> and familial hypercholesterolemia,<sup>26</sup> we concluded by 1973 that the liver controlled cholesterol homeostasis. If this were true, the corollary was that hepatic replacement would cure familial hypercholesterolemia. Consequently, we listed familial hypercholesterolemia from 1973 on as an indication for liver transplantation. However, the proposal was initially considered to be so irrational that it could not be acted on until more than a decade later.<sup>45</sup>

The reason was that our hypothesis of hepatic cholesterol control was seemingly discredited by the discovery of Goldstein and Brown<sup>46</sup> at Southwestern University (Dallas, TX) that fibroblasts and all cells in patients with familial hypercholesterolemia were deficient in the LDL receptors to

which circulating cholesterol-rich complexes are bound in the process of elimination. It was not possible to reconcile this fundamental discovery with the circumstantial evidence of hepatic cholesterol homeostasis obtained from the auxiliary liver and Eck fistula investigations. Clarification finally came from a series of reports from Goldstein and Brown between 1977 and 1981 (summarized in reference 47) showing that a large proportion of binding of cholesterol complex occurred in the liver.

Residual doubts about the liver's role in cholesterol homeostasis were removed dramatically on Valentine's Day 1984, when a 6-year-old patient of Goldstein and Brown who had irreversible cardiac damage from the premature atherosclerosis of homozygous familial hypercholesterolemia underwent combined heart (by Henry T. Bahnson) and liver (by Byers Shaw) transplantation at the University of Pittsburgh.<sup>48</sup> The girl's serum cholesterol, which had been 1,000 mg/dL, fell to 250 mg/dL within a few days<sup>49,50</sup> and remained at this level until her death 6.5 years later. For their elucidation of cholesterol metabolism, including its hepatic modulation in disease and health, Brown and Goldstein were awarded the Nobel Prize in December 1985.

### Control of Liver Volume and Regeneration

The essence of the double liver models (Fig. 2B–2D) that derived from the auxiliary liver transplant preparation (Fig. 2A) was division of the animal's own liver into competing fragments in which one liver fragment dominates the other by its avid clearance of hepatotrophic substances delivered through the portal blood supply (see previous section). With the final model (Fig. 2D), both liver fragments were detached from any portal venous inflow. Test substances were then infused into either the right or left portal vein branch. Seven growth factors in addition to insulin have been shown to prevent completely the changes caused by Eck fistula in the infused (but not the other) liver fragment.<sup>51</sup> In addition, two agents were found to have an opposite (antihepatotrophic) action (Table 2). These molecules in various combinations under different circumstances can profoundly affect liver structure, volume, and the capacity for regeneration in addition to hepatic function.

**Table 2. Growth Factors Revealed by Studies by the Eck Fistula Double-Fragment Model Shown in Figure 3D**

<b>Table 2. Growth Factors Revealed by Studies by the Eck Fistula Double-Fragment Model Shown in Figure 3D</b>
Stimulatory
Hormones
Insulin
Growth factors:
Cytosol substrate and ALR
Insulin-like growth factor II
Transforming growth factor- $\alpha^*$
Hepatic growth factor*
Immunosuppressants
Cyclosporine
FK 506 (Tacrolimus)
Immunophilins
FK 506-binding protein (FKBP <sub>12</sub> )
Inhibitory
Growth factors
TGF- $\beta$ †
Immunosuppression
Rapamycin†
NOTE. Discoveries were made between 1975 and 1995 (see reference 51 for annotation).
*Mitogenic in tissue culture.
†Inhibitory in tissue culture.

Because only two of the eight hepatotrophic factors (transforming growth factor  $\alpha$  [TGF- $\alpha$ ] and hepatocyte growth factor [HGF]) stimulate mitoses in tissue culture, the hepatotrophic effects of the other six had not been recognized with in vitro techniques. For hepatologists and immunologists as well as for transplant surgeons, it was particularly significant that the T-cell-directed immunosuppressants cyclosporine and tacrolimus are hepatotrophic, whereas rapamycin and transforming growth factor  $\beta$  (TGF- $\beta$ ) make up the short list of potent antihepatotrophic factors. The obvious implication of potential immunological modulation in the control of hepatic volume and regeneration has only begun to be explored.<sup>52,53</sup>

The most elusive of the six "occult" growth factors that are nonmitogenic in tissue culture was the molecule called "augmenter of liver regeneration" (ALR), which is found in regenerating rat<sup>54</sup> and dog<sup>28,55</sup> livers after partial hepatectomy and in the hyperplastic livers of weanling rats.<sup>51,54,56</sup> The purification of a crude hepatic cytosol<sup>28</sup> to >800,000<sup>51</sup> and cloning after 16 years of effort in our laboratories<sup>56</sup> added another important component to the complex network of modulators, both

stimulatory and suppressive, which regulate hepatocyte proliferation and hepatic regeneration at the organ level. ALR is a unique, heat-stable peptide whose gene shows 50% homology with the dual-function nuclear gene ERV1 of the yeast *Saccharomyces cerevisiae* (baker's yeast).<sup>57</sup>

The ERV1 gene is required for oxidative phosphorylation (respiratory chain) by the yeast and also is essential for mitosis, which ceases in 3 to 4 days after gene deletion.<sup>57</sup> If, as we believe, ALR is the mammalian homologue of ERV1, it is apt to be a major growth-regulatory gene, including at the organogenesis level. After the discovery of the ALR gene in the rat,<sup>56</sup> we identified the mouse and human ALR genes (and the protein products).<sup>58</sup> These were found in all three mammalian species to be highly conserved and preferentially expressed in the liver and the testis.<sup>56,58</sup>

The ALR gene maps to the mouse chromosome 17, in a region systemic with human chromosome 16, where the allele-rich T/t region involved in spermatogenesis is located.<sup>58</sup> Thus the physiological properties of the ALR protein almost certainly are not limited to liver regeneration. We have suggested involvement of ALR in the synthesis or stability of the nuclear and mitochondrial transcripts that are present in actively regenerating cells, particularly the germ cells of the testis.<sup>58</sup> The fact that so little is yet known about ALR compared with the other hepatotrophic factors makes this gene and its peptide inviting targets for basic and clinical inquiries. The potential value of ALR in the treatment of fulminant hepatic failure or in artificial liver support systems has yet to be determined.

## Anatomic Research

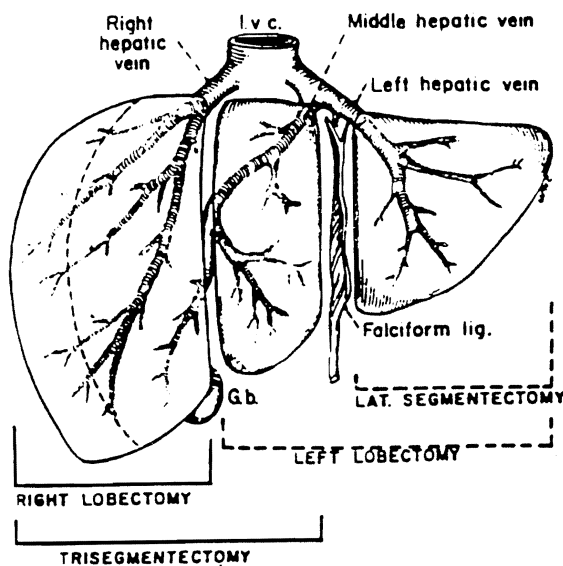
As a result of liver transplantation, interest was awakened in "anatomic trivia" such as the blood supply of the extrahepatic bile ducts, variations of the arterial and venous systems of the liver, and biliary tract anomalies that mandated modifications of standard transplant surgery. Knowledge of intrahepatic anatomy also assumed new clinical relevance.

The surgical-anatomic units that theoretically could be removed with partial liver resections had been incrementally clarified between 1927 and 1957 by the anatomic research of McIndoe and Counsellor,<sup>59</sup> Hjortsjo,<sup>60</sup> Healey,<sup>61</sup> Healey and Schroy,<sup>62</sup> Couninaud,<sup>63</sup> and Goldsmith and Woodburne.<sup>64</sup> Until the 1970s, however, only three

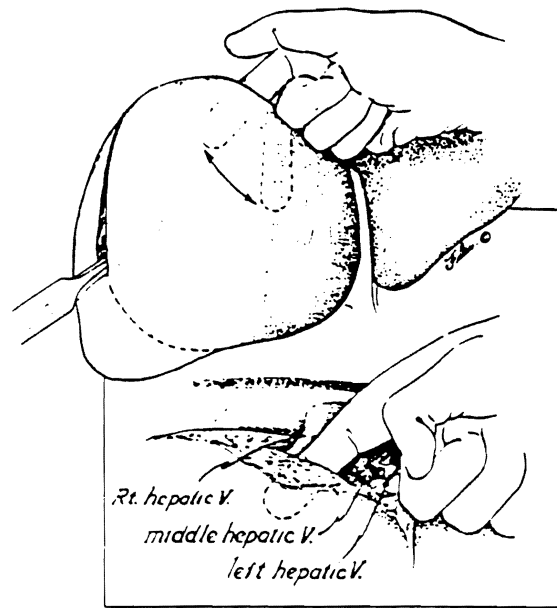
standard procedures had exploited this information for "controlled resections" in which the target specimens were devascularized in the hilum before entering the liver parenchyma: right and left lobectomy and left lateral segmentectomy (Fig. 4).

A fourth operation in which all of the liver to the right of the falciform ligament was removed (approximately 80%–85% of the organ) had been reported between 1951 and 1953 by Wangenstein,<sup>65</sup> Lortat-Jacob and Robert,<sup>66</sup> and Quattlebaum.<sup>67</sup> Enthusiasm for performance of this procedure (right trisegmentectomy, also known as extended right lobectomy<sup>68</sup>) had been dampened by an unacceptably high mortality rate (up to 40%).<sup>69-71</sup> Lack of concentrated experience with the difficult operation was the reason.

This changed when patients began to be referred to the University of Colorado in the late 1960s as candidates for orthotopic liver transplantation because of "unresectable hepatic tumors." Many of these malignancies could be removed instead with right trisegmentectomy. By 1974, 14 such patients had undergone the nontransplantation option with no surgical mortality.<sup>72</sup> Safe performance of right trisegmentectomy required an understanding of the anatomy in the umbilical fissure that is encoun-



**Figure 4.** The four types of partial hepatectomy most commonly performed with hilar control. Note that there are only four common resections: right and left lobectomy, right trisegmentectomy, and left lateral segmentectomy. (Reprinted with permission from Surg Gynecol Obstet 1975;141:429-438.)



**Figure 5.** Separation in the coronal plane of the anterior from the posterior segment of the right lobe during left trisegmentectomy after revascularization of the left lobe. Note that the dissecting finger is kept anterior to the right hepatic vein, the left and middle hepatic vein having been ligated or sutured. (Reprinted with permission from Surg Gynecol Obstet 1982;155:21-27.)

tered in dissecting the left branches of the portal triad. When 17 more cases were accrued with no deaths,<sup>73</sup> the procedure became a standard service.

Left trisegmentectomy, a fifth variety of controlled hepatic resection, also emerged from the liver transplantation clinics.<sup>74</sup> This operation involves removal of the true left lobe of the liver in continuity with the anterior segment of the right lobe plus part or all of the caudate lobe. The dangerous phase of left trisegmentectomy, during the development of the coronal plane between the anterior and posterior segments of the right lobe (Fig. 5), is facilitated by cross-clamping of the portal triad (the Pringle maneuver). The resulting normothermic ischemia of the residual liver fragment, which previously was considered acceptable for only 10 or 20 minutes, has been shown to be well tolerated for an hour or more by Huguet et al,<sup>75</sup> a liver transplant surgeon who trained at the University of Colorado.

Other liver transplant-derived strategies have been applied to hepatic resection, including in situ cooling of the totally devascularized liver, with or without venovenous bypass. In the opposite trans-

fer of technology, the increasingly sophisticated resection techniques that evolved largely from liver transplantation experience have contributed to the safety of the partial liver transplantation techniques pioneered by Henri Bismuth (Paris), Rudolf Pichlmayr (Hanover), Stephen Lynch and Russell Strong (Brisbane), and Christoph Broelsch (Chicago, Hamburg). Most recently, the brilliant studies of Strasberg<sup>76</sup> have shown that the science of pure gross anatomy is far from dead.

### The Biology of Cancer, With Particular Reference to Posttransplantation Lymphoproliferative Disorders

To succeed with organ transplantation, it is necessary to alter drastically the immune system of the recipient and thereby reduce one of the natural defenses against malignant tumors. By the late 1960s, there was evidence that the loss of tumor surveillance in immunosuppressed organ recipients could result in (1) accidental engraftment of donor malignancies, (2) accelerated growth of microscopic metastatic neoplasms of either donor or recipient origin, and (3) increased incidence of *de novo* malignancies.<sup>77,78</sup> Although real, the risks from the first two complications have been minimized by appropriate donor and recipient screening.

In contrast, a striking increase in *de novo* malignancies called posttransplantation lymphoproliferative disorders (PTLD) has occurred, particularly after the advent of the T-cell-directed agents cyclosporine,<sup>79,80</sup> tacrolimus,<sup>81</sup> and OKT3.<sup>82</sup> PTLD resemble the malignancies found in immune deficiency states,<sup>83-85</sup> including acquired immunodeficiency syndrome.<sup>86</sup> Originally designated "reticulum cell sarcomas,"<sup>87</sup> they constitute a spectrum of lymphoproliferative neoplasms, of which most are B-cell lymphomas<sup>88-90</sup> that are highly but not invariably associated with Epstein-Barr virus (EBV) infestation.<sup>91-93</sup> Because they are dramatically subject to immune surveillance,<sup>80</sup> the pathogenesis and treatment of these lesions have been of special interest to tumor biologists as well as to clinicians.

### Pathogenesis of PTLD

#### Immune Suppression

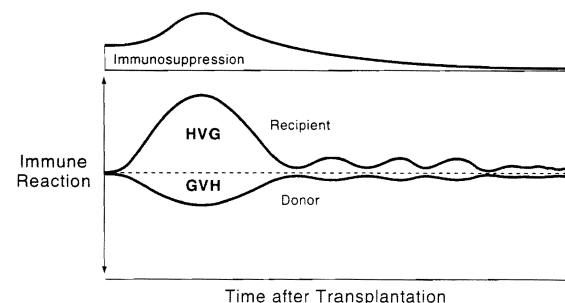
Heavy immunosuppression, either applied by protocol or in a management response to rejection,

usually precedes the appearance of PTLD. Conversely, dose reduction or discontinuance of such treatment frequently is followed by PTLD regression.<sup>80</sup> This is not necessarily followed by rejection,<sup>80,94</sup> even when treatment is permanently stopped,<sup>95</sup> a finding that cannot be accounted for by the simple loss and recovery of immune surveillance.

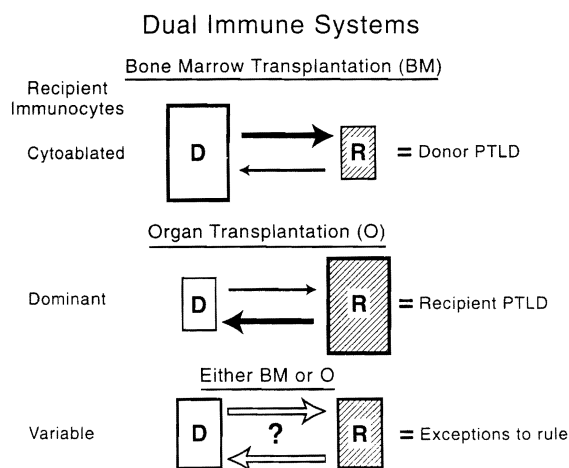
### Chimerism and Tumor Origin

We have suggested that organ acceptance under immune suppression involves the interaction of a persistent small population of migratory ("passenger") donor leukocytes from the allograft (microchimerism) with the leukocytes of the recipient immune system (the two-way paradigm)<sup>96-98</sup> (Fig. 6). The two-way immune reaction in organ recipients consists of low-grade graft-versus-host (GVH) and host-versus-graft (HVG) components. PTLD can be viewed as a pathological deviation from this joint immunocyte activation, to which powerful cofactors are added that act on both populations.

Because host leukocytes in most organ recipients vastly outnumber the chimeric donor cells, the clinical PTLD are usually of recipient origin<sup>99-101</sup> (Fig. 7). After bone marrow transplantation, in which the leukocyte proportions are mirror image, almost all PTLD are of donor origin (Fig. 7). The occasional exceptions to this generalization usually are seen during the early posttransplantation period, during which heavy immune suppression is administered.



**Figure 6. Contemporaneous HVG and GVH reactions in the two-way paradigm of transplantation immunology.** After the initial interaction, the evolution of nonreactivity of each leukocyte population to the other is seen as a predominantly low-grade stimulatory state that may wax and wane rather than a deletional one.



**Figure 7. Recipient cell populations and their relationship to PTLD phenotype after conventional bone marrow versus organ transplantation (see text).**

### Viral Factors

The strong association of human PTLD with EBV infection has been known for more than 15 years.<sup>80,91-93</sup> This DNA virus is recognized by cytotoxic T cells (CTL) whose surveillance of EBV in infected B cells<sup>102-106</sup> may be facilitated by an accessory nonspecific contribution of natural killer (NK) cells.<sup>107-109</sup> This defense mechanism explains why the vast majority of normal humans harbor EBV but do not develop recurrent infectious mononucleosis or B-cell neoplasms. Under immune suppression, however, the EBV-infected immunoblast is freed from T-cell surveillance, an event of particular importance in the development of PTLD because of the uniquely potent B-cell-transforming quality of the virus.

The cumulative effect of a double lymphoproliferative drive (i.e., chimerism plus EBV infection) in the absence of immune suppression has been shown in the South American cotton-top tamarin primate species (*Saguinus oedipus*), in which birth normally is of dizygotic twins. Placental fusion routinely leads to multilineage chimerism,<sup>110,111</sup> similar to that seen in Freemartin cattle.<sup>112</sup> These chimeric tamarin have fully normal immunological reactivity, including alloantigen-driven proliferative responses and generation of allogeneic specific CTL precursor cells, and they show in vitro evidence of mutual immunological nonreactivity of the coexisting cell populations<sup>110,111</sup> and reciprocal acceptance of skin grafts.<sup>113</sup> The addition of an EBV infection causes severe lymphoproliferative dis-

ease, including unequivocally malignant neoplasms.<sup>114-116</sup>

Approximately 8% of clinical posttransplantation lymphomas in humans develop in the absence of EBV infection.<sup>117</sup> Because these tumors also may involute with reduction or discontinuance of immune suppression,<sup>118</sup> they extend the possibility of therapeutic immune modulation beyond the EBV epitope.

### Treatment Algorithms for PTLD

PTLD occur in all kinds of organ recipients, but the highest incidence has been after liver transplantation. The risk can be reduced at the outset by avoiding the use of antithymocyte globulin (ATG) and OKT3 in conjunction with other T-cell-directed agents (i.e., cyclosporine and tacrolimus), except as a last resort. The most effective treatment of PTLD is discontinuance or drastic reduction of immune suppression. The type of allograft influences the aggressiveness with which this strategy is pursued. If a transplanted kidney is allowed to be rejected, the PTLD will disappear in essentially all cases, allowing drug-free return to dialysis. There is little merit in renal recipients in pursuing cyclic gyrations of immunosuppressant dosage in response to tumor growth on one hand and rejection on the other.

Persistent efforts at immunosuppressant dose control rather than complete discontinuance are more justifiable in liver, heart, and lung recipients for whom "treatment rest" and retransplantation may not be feasible. In our pediatric liver transplant program, histopathologically verified PTLD was diagnosed in 28 (12.1%) of the 232 consecutive primary allograft recipients treated with tacrolimus from 1989 through 1994 (Table 3). Although 5 of the 28 died of PTLD-related complications, patient and graft survival (82.2% at 4 years) are essentially the same as in the non-PTLD group. In addition to careful monitoring for EBV infection, management of the PTLD has been facilitated by our policies of arbitrary gradual tacrolimus dose reduction and acceptance of lower plasma and blood levels with the passage of time in addition to early discontinuation of prednisone therapy (Table 3) and avoidance of adjunct agents, including OKT3 and azathioprine.

Although complete discontinuation of immunosuppression can result in PTLD involution without rejection of the transplanted organ, reluctance to



**Table 3. Primary Liver Transplantation (n = 232) in Children Receiving Tacrolimus-Based Immune Suppression during 1989-1994**

	Months of Follow-up				
	3	12	24	36	48
Survival (%)					
Patient	90.2	86	85	84	84
Graft	83	79.2	78.2	77.3	77.3
Tacrolimus (mean)					
Dose (mg/d)	5.6	3.3	2.9	2.9	2.4
Plasma Concentration* (trough ng/mL)	0.84	0.56	0.43	0.4	0.24
Prednisone (%)					
None	79	82	88	93	91
≤5	9	13	7	5	7
≥5 mg/d	12	5	5	2	2

NOTE. N = 232. Age,  $5.0 \pm 5.2$  (mean  $\pm$  SD; median, 2.7) years. Mean follow-up,  $44 \pm 14.6$  months. 28 patients developed PTLT; 23 (82%) survive with their original graft. Principal sites were gastrointestinal (9); lymph nodes (8); liver (6); spleen (2); and tonsil, skin, and blood (leukemia) (1 each).  
\*For approximate whole blood values, multiply  $\times 10$ .

jeopardize the graft is a psychological barrier to taking this drastic step. Moreover, a response may not be seen even with drug discontinuation if prior immune suppression has been so severe that recovery of natural surveillance cannot occur in time to overtake a rapidly growing neoplasm. Consequently, early multiple-agent lymphoma therapy has been recommended if PTLT regression is not seen within a few days.<sup>119-122</sup> Because the lymphoma protocols call for high doses of drugs that are themselves immunosuppressive, their use variably contravenes the restoration of host immune surveillance, upon which survival ultimately depends (Fig. 8). In addition, the opportunity may be lost for a systematic search for an appropriate reduced dose of conventional immunosuppression that may allow PTLT control without rejection of the graft.

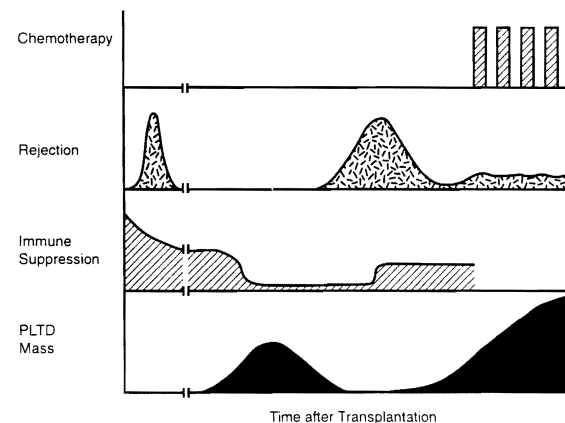
Recent developments in cellular immune modulation for bone marrow transplantation have suggested other options. In bone marrow recipients, regression of EBV-positive PTLT of donor phenotype has been accomplished by infusion of naive cytotoxic T lymphocytes obtained from the original donor.<sup>123</sup> In an attempt to minimize the attendant risk of GVHD, sorted EBV-specific CTL have been used, with arrest of early lymphoproliferation

in EBV-infected bone marrow recipients at high risk from infectious mononucleosis syndromes.<sup>124</sup>

Naive recipient leukocytes with which to apply the same principles to organ transplantation PTLT in mirror image are almost never available. This need could be met in three ways: (1) cryopreservation of pretransplantation peripheral blood mononuclear cells (PBMC) or bone marrow, (2) use of a surrogate leukocyte donor HLA identical to the recipient,<sup>125</sup> or (3) functional resurrection of the recipient's own PBMC or bone marrow.

We have had experience with the third option in liver, lung, heart, and kidney recipients 2 months to 12 years posttransplantation.<sup>118</sup> PBMC were obtained by leukopheresis, depleted of leukocytes of monocyte/macrophage lineage, and cultured in the presence of interleukin 2 for 10 to 11 days. Although infusions of the resulting LAK cells caused regression of EBV-positive tumors in all four such recently reported cases, efficacy could not be evaluated in the EBV-negative cases because of confounding circumstances.<sup>118</sup>

It must be emphasized that because the LAK cells are lineage committed and not self-renewable, they provide only a bridge to the ultimate objective of natural recipient surveillance. The principal value of such treatment will be in those patients who are so deeply immunosuppressed that they



**Figure 8. Prototypic error of giving lymphoma chemotherapy (which is inherently immunosuppressive) to a liver transplant recipient whose recovery from PTLT depends on restoration of immunological tumor surveillance. The abdominal PTLT mass that involuted after reduction of immune suppression regrew with restoration of therapy. The error was compounded by initiation of CHOP lymphoma treatment, after which discontinuance of tacrolimus and prednisone was no longer effective.**

cannot recover natural immune surveillance promptly. Retardation of immunological recovery with chemotherapy or high-dose radiotherapy works at cross-purposes with the global strategy of surveillance restoration and should be used, if at all, only when all else has failed.

### *Is Immunization Possible for PTLT?*

Prior vaccination of cotton-top tamarins with either EBV or its recombinant proteins protects the animals against subsequent development of EBV-induced malignant lymphomas.<sup>126-128</sup> Such an approach might be used to immunize EBV-negative human organ allograft recipients who are at predictably high risk from EBV-associated PTLT (e.g., liver, intestinal, or multivisceral transplant candidates<sup>129</sup>). The immunity of EBV-seropositive patients might also be boosted by vaccination with autologous irradiated EBV-transformed B-cell blasts.

As with other vaccination protocols, repeated exposure to the antigen may be required to attain a protective level of immunization. Because vaccination of EBV-negative transplant candidates could have a theoretical risk of accidental transplantation of autologous EBV-exposed antigen-presenting cells, immunization of these patients should be with recombinant EBV peptides only. However, patients who are already EBV seropositive could have their immunological memory to EBV-transformed cells boosted with irradiated EBV-transformed cells. This has been shown to be feasible in mouse experiments without a risk of SV<sub>40</sub> tumor engraftment.<sup>130,131</sup>

### **Conclusions**

Our fledgling Journal can be vitalized from the same wellsprings that have been tapped but not exhausted in the evolution of liver transplantation to its present state. Examples with historical roots that continue to generate interest have been described. These could be categorized as physiological/metabolic, anatomic/technical, and oncological. However, no effort has been made to describe the full spectrum of potential clinical and basic scientific subjects that could be considered by the editors for inclusion.

For example, no mention has been made of the virtually limitless areas of infectious disease or of xenotransplantation. The role of liver transplantation in exposing the meaning of immunological

tolerance and how to achieve it also is too large to briefly summarize. It would be equally futile to dwell on the advances in pharmacological immune suppression of the last two decades other than to note that these were driven primarily by clinical research designed to improve liver transplantation.<sup>79,132-140</sup> Management improvements were then adapted with only minor variations for transplantation of the kidney, heart, and other organs.

No one understands the need for diversification of articles better than the editors of *Liver Transplantation and Surgery*. The key word for advancement of this objective is "liver," rather than either "transplantation" or "surgery." This insight notwithstanding, the editors will be powerless to shape and strengthen the Journal without receipt of fine original manuscripts. The prime responsibility for this rests at present with liver transplant surgeons, hepatologists, and anesthesiologists. Because the Journal was their brainchild, failure to broaden its base beyond transplantation will redound to the discredit of these elite but relatively small corps of specialists.

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